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THE EFFECTS OF SAPROPEL EXTRACTS ON THE ACTIVITY OF AMYLOLYTIC ENZYMES APPLIED IN ETHYL ALCOHOL PRODUCTION

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In ethyl alcohol production, amylolytic enzymes such as amylases and glucoamylases play a crucial role. However, the activity of these enzymes depends on different factors such as pH, temperature, and ionic conditions of the environment. Therefore, the application of sapropel extracts in ethyl alcohol production can affect enzymatic activity. The prospective application of sapropel extracts as an alternative to antibiotics was suggested by [1] to replace penicillin G and virginiamycin, which are currently the most antibiotics used in ethanol production [2].

It was previously found that preparations of sapropels have a significant bactericidal effect [3–5]. Sapropel extracts are prepared by ultrasonic cavitation of sapropels. Due to the presence of humic substances in Sapropel extracts, these extracts may arrest bacterial and fungal growth and contain microbial enzyme activation via metallic ions contained in humic substances in an active organic form, particularly Cu, Fe, and Zn. Additionally, lipids contained in humic substances may prevent nutrient uptake by fungal spores and later germination [6, 7].

Therefore, this research aims to study the effects of Sapropel extracts based Humic substances on thermostable α-amylase: *Bacillus licheniformis* α-amylase (BLA) used in ethyl alcohol production.

The objects of the study are Sapropel extracts obtained in the RAS Limnology Institute with alkaline extraction and ultrasound treatment of air-dry sapropels from the Seryodka deposit (Pskov region, Russia). Sapropel extracts used were obtained from hot method extraction at 40 °C (104 °F) at pH 11.8 and 4.5 % concentration of dry

matter and 30 % concentration of Humic substances (HS). Enzyme preparation Distizym BAT-special (Erbsloh, Germany), which contains *Bacillus licheniformis* α-amylase (BLA).

The activity of α-amylase activity was studied at 30 °C and 70 °C. Different doses (10; 25 and 50 µl) of sapropel extracts, pH 4.5; 7 and 9.5 were added to enzymes. The activity of α-amylase enzymes was determined according to GOST (GOST-54330, 2011) with some modifications.

The highest activity of *Bacillus licheniformis* α-amylase (1025 units AS/ml) was observed at 30 °C when 50 µl of sapropel extracts, pH 4.5, was added to enzymes. This activity is 40 % more compared to the control (731 units AS/ml). The lowest activity (680 units AS/ml) was obtained when 50 µl of sapropel extracts, pH 7, was added to *Bacillus licheniformis* α-amylase (BLA) while at pH 9.5, the activity was 878 units AS/ml. When the temperature was increased to 70 °C, the activity was decreased (702 and 820 units AS/ml for control and experimental, respectively), but the highest impact was at 30 °C. This difference may be due to the activity of humic acids, which depends on temperature. According to [8], Humic Acids (HS) retain their properties at 40 °C, which is closer to 30 °C than 70 °C.

The results of this study showed that the effects of Sapropel extracts on the activity of *Bacillus licheniformis* α-amylase depend on the dose, pH of sapropel extracts, and the temperature. The results of this research work will allow the application of sapropel extracts, at optimum conditions, in the production of ethyl alcohol technology.

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